Novobiocin appears to be the first recorded example of the natural occurrence of a carbamate ester of a sugar derivative.

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THE COMBUSTION OF CARBON SUBNITRIDE, C_4N_2, AND A CHEMICAL METHOD FOR THE PRODUCTION OF CONTINUOUS TEMPERATURES IN THE RANGE OF 5000–6000°K.[1]

Sir:

A flame temperature of slightly over 5000°K. was reached by combusting cyanogen with oxygen under pressure.[2] The high temperature is due to the high endothermic heat of formation of cyanogen (−73.60 kcal./mole gas at 25°) and the great thermal stability of the combustion products, *i.e.*, CO and N_2.

It was obvious that if a compound existed with a still higher endothermic heat of formation than cyanogen, which also could be combusted to CO and N_2, higher flame temperatures could be attained. A whole series of such compounds exist. They are the dinitriles of acetylene-dicarboxylic and polyacetylene-dicarboxylic acids or dicyanoacetylene and dicyano-polyacetylenes. Their general structural formula is $N:C\cdot(C:C)_n\cdot C:N$.

The first member of the series ($n = 1$) or C_4N_2 was isolated by Charles Moureu and Jacques C. Bongrand[3] and named carbon subnitride.

C_4N_2 is a water-white liquid with a b.p. 76.5°, m.p. 20.5° and d^{25}_4 0.9703. It has a high endothermic heat of formation, −149.81 kcal./mole gas at 25°, and can explode to carbon powder and nitrogen. The subsequent members have not been described in the literature, but the dicarboxylic acids, from which they are derived, have been known since A. v. Bayer's[4] classical researches.

A still higher temperature can be obtained by combining an endothermic fuel with an endothermic oxidizer; in the above case the obvious substitute would be ozone for O_2. Dr. A. Streng recently succeeded in measuring flame velocities of pure O_3–O_2 mixtures in the range of 17–55 mole% O_3 and successfully burned such mixtures with H_2, CO and $(CN)_2$.

Since the calculated temperature of the $(CN)_2$–O_2 flame has been checked experimentally,[5] the enthalpy data for CO and N_2 can be used with confidence to calculate[6] the temperatures of $C_4N_2 + O_2$ and $+O_3$ flames at various pressures. They are as follows, in °K. (± 2°), for the reaction mixture specified:

	Temperature °K.		
Atm.	1.0	10	40.82 (= 600 psia)
$(C_4N_2)_g + 2O_2 \longrightarrow 4CO + N_2$; $\Delta H_{298°} = +254.6$	5261	5573	5748
$(C_4N_2)_g + 4/3\ O_3 \longrightarrow 4CO + N_2$; $\Delta H_{298°} = +299.9$	5516	5936	6100

Temperature decrease due to ionization of the combustion products can be neglected completely even at 6000°K.

C_4N_2 was burned with oxygen in both a diffusion and premixed flame in the range of $2O_2$–$4O_2$ per mole C_4N_2. The nitride burns with a bright white-blue flame similar in many respects, but *not* identical, to the $(CN)_2$–O_2 flame. The $C_4N_2 + 2O_2$ flame burns to only CO and N_2 and has a calculated temperature, as outlined previously, of 5260°K. An oxygen-rich flame, of the composition $C_4N_2 + 2.40\ O_2$ produced, in addition to CO, CO_2 and N_2, the nitrogen oxides, NO_2, N_2O_3 and mainly NO; calculated as NO, their amount equalled 0.6–0.9 mole% of the combustion products.[7]

(1) This research was supported by the United States Air Force through the Air Force Office of Scientific Research of the Air Research and Development Command under Contract No. AF 18(600)-1475.

(2) J. B. Conway, W. F. R. Smith, W. J. Liddell and A. V. Grosse, THIS JOURNAL, **77**, 2026 (1955).

(3) C. Moureu and J. C. Bongrand, *Bull. soc. chim.*, [V], 846 (1909); *Ann. Chim.*, **14**, 5 (1920).

(4) A. v. Bayer, *Ber.*, **18**, 678, 1885, 2269 (1885).

(5) J. B. Conway, R. H. Wilson, Jr., and A. V. Grosse, THIS JOURNAL **75**, 499 (1953).

(6) Acknowledgment is due to Drs. Rapp and Irgon of Reaction Motors, Inc., for their accurate calculations. N.A.C.A. tables were used up to 6000°K.; above 6000°K. the data were extrapolated.

(7) Our experimental combustion and analytical techniques and results are fully described in Technical Note No. 1 (A. V. Grosse and A. D. Kirshenbaum) Report Control No. AFOSR-TN-56-13, Contract No. AF 18(600)-1475, Project No. 7-7968, dated December 15, 1955, U. S. Air Force, Office of Scientific Research, Air Research & Development Command, P. O. Box 1395, Baltimore 3, Md.

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A NEW ATP-FORMING REACTION: THE REDUCTIVE DEAMINATION OF GLYCINE

Sir:

Extracts of the amino acid-fermenting organism, *Clostridium sticklandii* (strain HF), catalyze the formation of acetic acid and ammonia from glycine when 1,3-dithiolpropanol (DTP) is added as the reducing agent.[1] Decomposition of glycine-2-C^{14} in this system results in the formation of acetic acid labelled exclusively in the methylene carbon atom.[2] Aged preparations derived from extracts of alumina-ground dried cells exhibit dependencies on DPN and Mg^{++} (Table I). There also appears to be a requirement for pyridoxal phosphate, particularly in the absence of added DPN, but this is somewhat variable. When more highly purified enzymes prepared from sonic extracts are employed, there is almost complete dependency on orthophosphate and an adenylate nucleotide (Table II). Since AMP, ADP and ATP are equally efficient in promoting the reaction, the preparation undoubtedly contains an adenylate kinase. Arsenate substitutes for phosphate and eliminates the need for an acceptor nucleotide.

The stoichometry of the over-all process as determined by direct analysis of the reaction products

(1) T. C. Stadtman, *3rd Int. Congr. Biochem.*, Bruxelles, p. 53 (1955).

(2) C^{14}-acetate was recovered by steam distillation, checked for purity by Duclaux distillation and degraded using the Schmidt reaction according to E. F. Phares, *Arch. Biochem. and Biophys.*, **33**, 173 (1951).

TABLE I

COFACTOR REQUIREMENTS FOR THE REDUCTION OF GLYCINE-2-C^{14} TO ACETATE-2-C^{14}

The complete system contained tris-(hydroxymethyl)-aminomethane (TRIS) buffer, (*p*H 8.7) 25 μmoles; $MgSO_4$, 3 μmoles; DPN, 0.2 μmole; pyridoxal phosphate, 0.006 μmole; DTP, 20 μmoles; 0.2 μC 2-C^{14}-glycine (*ca.* 30,000 cts./min.), 10 μmoles and 8 mg. protein. Reactants in 0.5 ml. final volumes were incubated anaerobically at 31° for two hours.

Omission	Acetate-2-C^{14} formed, cts./min.[3]
...	940
DPN	225
Mg^{++}	465

TABLE II

THE EFFECT OF ORTHOPHOSPHATE (PO_4), ARSENATE (AsO_4) AND ADENYLATE NUCLEOTIDES ON THE CONVERSION OF GLYCINE TO ACETATE

In addition to the reactants each sample contained TRIS buffer (*p*H 8.7), 20 μmoles; $MgCl_2$, 3 μmoles; DPN, 0.1 μmole; pyridoxal phosphate, 0.003 μmole; DTP, 9 μmoles; 0.2 μC glycine-2-C^{14}, 10 μmoles and 3.6 mg. protein (30 to 35% satd. $(NH_4)_2SO_4$ fraction), in a final volume of 0.5 ml. Incubations were carried out anaerobically at 31° for 90 min.

Experiment	Additions, μmoles	Acetate-2-C^{14} formed, μmoles[3]
1	None[a]	0.09
	PO_4 10, ADP 5, or AMP 5 or ATP 5	1.18 1.30 1.28
	PO_4 10, ADP 10	1.45
	PO_4 10, AMP 5, ATP 5	1.60
2	None[a]	0.25
	PO_4 10	0.56
	ADP 1	1.01
	PO_4 10, ADP 1	1.87
3	PO_4 5[a]	0.43
	AsO_4 5	1.29
	AsO_4 5, PO_4 5	1.42
	AsO_4 10	1.42
	AsO_4 10, AMP 5	1.40

[a] The enzyme preparation (not dialyzed) contained 0.88 μmole of orthophosphate per 3.6 mg. protein employed.

(Table III) shows that the reaction can be described by the equation

$$CH_2NH_2COOH + PO_4 + ADP + R(SH)_2 \longrightarrow CH_3COOH + NH_3 + ATP + RSS \quad (1)$$

The most significant result of these experiments is that for each mole of glycine converted to acetic acid and ammonia there is a concomitant esterification of one mole of orthophosphate which is incorporated into ATP.

Reduction of glycine in the presence of P^{32}-labelled orthophosphate and ADP (or AMP) results in a marked synthesis of P^{32}-labelled ATP. No labelled ATP is detected chromatographically[4] when glycine is omitted from the otherwise complete system or when amino acids such as citrulline, lysine or proline[5] are substituted. The failure to observe any phosphorylation associated with the reduction of proline to δ-aminovalerate by DTP,[1] a reaction also catalyzed by this enzyme fraction, suggests that the phosphorylation associated with glycine reduction is not solely the result of dithiol oxidation.

TABLE III

GLYCINE REDUCTION BALANCE EXPERIMENTS

Reaction mixture components as in Table II with AMP, 5 μmoles and K_2HPO_4, 3–10 μmoles. The enzyme preparation used to measure NH_3 formation had been precipitated with satd. Na_2SO_4 and redissolved in buffer to lower its $(NH_4)_2SO_4$ concentration. The amount of DTP oxidized was measured in incubation mixtures reduced to one-half the usual volume; all components were added in proportionally smaller amounts except for enzyme and glycine.

Glycine[6] dec., μmoles	$(SH)^7$ oxid., μ equiv.	PO_4[8] uptake, μmoles	P_{10} min. formed, μmoles	Acetate[2,9] formed, μmoles	NH_3[10] formed, μmoles
0.85	[b]	0.71	0.89	1.03[a]	0.77
1.01	[b]	1.07	0.98	1.01	1.23
[b]	2.52	1.25	0.89	1.21	[b]
[b]	2.30	0.98	0.98	1.0	[b]

[a] Glycine, 5 μmoles, present.
[b] Not measured.

The balance data presented, taken together with the fact that there is a compound formed that can be arsenolyzed, make it evident that the reductive deamination of glycine must involve the formation of a high energy phosphorylated intermediate. An unstable glycine derivative that is accumulated by another enzyme fraction derived from *C. sticklandii* may furnish a clue as to the nature of this intermediate.

(3) Residual C^{14}-glycine was removed by treatment with Dowex-50-H^+ resin at *p*H 1–2 and aliquots of the supernatant solutions assayed for C^{14} after neutralization. Identity of the radioactive product was established by steam distillations and Duclaux distillations.

(4) L. V. Eggleston and R. Hems, *Biochem. J.*, **52**, 156 (1952).

(5) T. C. Stadtman, *J. Bact.*, **67**, 314 (1954).

(6) E. C. Cocking and E. W. Yemm, *Biochem. J.*, **58**, xii (1954).

(7) P. D. Boyer, THIS JOURNAL, **76**, 4331 (1954).

(8) C. H. Fiske and Y. SubbaRow, *J. Biol. Chem.*, **66**, 375 (1925).

(9) Acetate was also estimated as acethydroxamate after incubation with the acetyl kinase system of I. A. Rose, M. Grunberg-Manago, S. R. Korey and S. Ochoa, *ibid.*, **211**, 737 (1954).

(10) Ammonia was measured by direct nesslerization of perchloric acid filtrates.

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SYNTHESIS OF COMPOUNDS RELATED TO RESERPINE. CONVERSION OF AN INTERMEDIATE TO 16-METHYLYOHIMBANE

Sir:

In the course of investigating the relationship of structure to reserpine-like activity we have synthesized a number of derivatives related to reserpine having appropriate substituents at carbon atoms 16 and 18 of the yohimbane skeleton. We now wish to report the total synthesis of 16-carbomethoxy-18-hydroxy-$\Delta^{15(20)}$-yohimbene which contains a double bond at a position suitable for manipulating the stereochemistry of the important D/E ring junction.

Treatment of methyl 2-carbomethoxy-3,4-dimethoxyphenylacetate[1] with chloromethyl ether and stannic chloride at 0° gave methyl 2-carbomethoxy-3,4-dimethoxy-6-chloromethylphenylacetate, m. p. 81–81.5° (found: C, 53.64; H, 5.41; Cl,

(1) C. Schöpf, U. Jäckh-Tettweiler, G. Mayer, H. Perrey-Fehrenbach and L. Winterhalder, *Ann.*, **554**, 77 (1940).